

Amendments to the Specification

Please replace paragraph [054] with the following amended paragraph:

[054] Column fractions containing the purified Tirt fusion protein were pooled, dialyzed into buffer A (50mM Tris-pH 7.5, 1mM EDTA, 1mM DTT, and 10% glycerol) using a microcon 30 membrane concentrator (Amicon, Beverly, MA), and then used to assay for RT activity. A highly sensitive product enhanced reverse transcriptase (PERT) assay was used to detect RT activity. The assay required the reverse transcription of a Brome Mosaic virus (BMV) RNA template to produce a small cDNA that was then further amplified by PCR (Fig. 5). Briefly, the assay was performed by first assembling the PCR amplification reaction mix in the bottom of a 0.2 ml tube containing: MgCl₂-free PCR buffer, 1X (Promega, Madison, WI), 1μM each BMV-PCR1 primer (5'-CGTGGTTGACACGCAGACCTCTTAC-3') [SEQ ID NO: 9] and BMV-PCR2 primer (5'-TCAACACTGTACGGCACCCGCATTC-3') [SEQ ID NO: 10], 0.8 mM each dNTP, and Taq polymerase (Promega). The RT reaction mix was then assembled on top after sealing the lower PCR reaction mix with a layer of wax using an Ampliwax pellet (PCR-Gem 50, Applied Biosystems, Roche). The RT reaction mix contained: RT buffer (50mM Tris-pH 8.3, 75mM KCl, and 10mM DTT), 2.5 mM MgCl₂, 0.17% NP-40, 10 units of RNasin (Promega, Madison, WI), 0.8mM each dNTP, 0.02μM RT primer (5'-GGTCTCTTTTAGAGATTTACAGTG-3') [SEQ ID NO: 11], 100ng of Brome Mosaic Virus (BMV) RNA (Promega), and a source of RT. The source of RT added to the reaction was either the purified Tirt fusion protein described above or commercially available Moloney Murine Leukemia Virus RT (MMLV-RT) (2 units). The reaction tube was then placed in a thermocycler under the following conditions: 1 cycle at 37°C for 1 hour (reverse transcription); 1 cycle at 94°C for 1 minute; 30 cycles at 94°C for 15 seconds, 56°C for 15 seconds, and 72°C for 15 seconds (amplification); and finally 72°C for 5 minutes. Amplified DNA was detected by electrophoresis of the reaction mix on a 5% polyacrylamide gel followed by staining with ethidium bromide.

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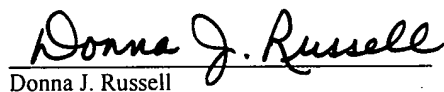
Should the Examiner have additional questions or concerns regarding this application, Applicants' representative may be reached by telephone at (615) 773-3583.

Respectfully submitted,



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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450 on February 4, 2007.


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